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Low Ester Pectin from Apple Pomace

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A simplified procedure is described for the preparation of low ester pectin from apple pomace, in which tomato pectase is used as the de-esterification catalyst. The essential steps in the process are polyphosphate extraction, filtration and concentration of the pectin extract, simultaneous de-esterification and starch removal, enzyme inactivation, and precipitation and purification of the product. The proposed process is rapid and easily controlled and requires only minor changes in the usual procedure for pectin manufacture.

A FEW years ago this laboratory undertook the development of a commercial process for preparing low ester pectin. Initial consideration was given to acid de-esterification, a method which had previously been studied by Baker and Goodwin (*4*). This method is time consuming, requires acid-resistant equipment, and causes some degradation of the product. Alkaline saponification was discarded after only slight consideration, because de-esterification by this method was accompanied by degradation. Alkaline de-esterification was used by McDowell (*13*), and, more recently, by McCready, Owens, and Maclay (*12*). This latter group reported that a low ester pectin useful for various purposes may be prepared by reacting pectin with dilute ammonium hydroxide at 15° C.

Pectase was considered a possible catalyst for the de-esterification of pectin because the milder conditions used with this catalyst would be expected to prevent the degradation incident to other methods. The initial results indicated that, although the product had a high molecular weight (as evidenced by high viscosity in aqueous solution), it produced calcium-pectinate gels inferior to those prepared from acid–de-esterified pectins (*9*, *20*). A study (*18*) to determine the cause of this anomalous behavior of the enzyme–de-esterified product confirmed the previous hypothesis that the enzyme, in contrast to acid, acts in a nonrandom manner. An investigation of the cataphoretic behavior showed clearly that the enzyme–de-esterified product was heterogeneous with respect to degree of esterification of the molecules within a given preparation, whereas the acid–de-esterified product and the nonde-esterified product were homogeneous in this respect. In view of the known variation in calcium optimum with progressive changes in degree of esterification (*9*), it is obvious that in a highly heterogeneous mixture not all the molecules of low ester pectin would be able to exert their maximum effect on gel strength at any given level of calcium concentration. It would be expected that, with a lower average degree of esterification, the heterogeneity of the product would be reduced and its calcium gelation behavior thereby improved. This was found to be the case. Other factors which led to improvement in the quality of the enzyme-prepared product are (*a*) less degradation because of milder conditions of extraction with polyphosphates, (*b*) minimized pectinase action through pH control, and (*c*) the minimized effect of calcium on dispersibility of the final product by recipitating at a pH of 4 or above.

The present paper describes a unified procedure as a basis for the large scale preparation of high quality enzyme–de-esterified pectin.

EXPERIMENTAL PROCEDURE

Various steps in the preparation of low ester pectin were investigated, with the threefold objective of (*a*) obtaining maximum yields of a high quality product, (*b*) efficiently utilizing the catalyst (tomato pectase), and (*c*) coordinating the separate steps into a unified process. These studies center around three steps—leaching, extraction, and the enzymic de-esterification process itself.

LEACHING. In the preparation of a liquid pectin concentrate from apple pomace, the pomace is usually leached with cold water before extraction to remove sugars, salts, and coloring matter. In the preparation of dry pectin this is less important because the precipitating agent removes most of these impurities. In the preparation of low ester pectins it is necessary to remove the polyvalent cations by washing with acidified alcohol. This procedure also removes coloring matter and sugars and thus eliminates the necessity for leaching before extraction.

It is not generally recognized that, in some cases, leaching apple pomace with cold water removes a considerable portion of pectin of a quality commensurate with that subsequently extracted. In order to determine the total amount of pectin extractable from apple pomace and the portion lost by leaching, a 200-gram sample of average quality pomace (ground to 4 mesh) was mixed with 15 parts by weight of distilled water at 50° C. After 15 minutes the leach water was drained through cheesecloth, and the pomace was extracted with 8 grams of a commercial sodium tetraphosphate at pH 3.4 and 90° C. for 50 minutes. The total weight of the mixture was 2400 grams. The pectin extract was removed by pressing. The press cake was extracted a second and then a third time in the same manner. The pectin concentration and jelly grade of each fraction were determined, and the dry pectin grade was calculated (Table I). Of the total extractable pectin (expressed as jelly units), 14% was lost in the leach water, and the pectin was of high quality. Small amounts of poor quality pectin were obtained in the second and third extracts.

EXTRACTION. Extraction of pectin from apple pomace by means of polyphosphates appeared to offer several advantages over the usual acid extraction method. As pointed out by Baker and Woodmansee (*5*), increased yields of pectin are obtained, and the mild conditions of acidity (pH 3.4 ± 0.2) reduce degradation of the product and lessen corrosion of equipment. A particular advantage in the preparation of low ester pectin by enzyme de-esterification is that the polyphosphate, serving as a calcium sequestering agent, prevents gel formation during enzyme treatment. The increased salt concentration also increases the activity of the enzyme.

Conditions for extraction were studied in laboratory scale experiments to determine the effects of time, temperature, pH, and quantity of extractant on yield and grade of pectin. (These studies were made prior to the discovery that large amounts of high grade pectin were lost by leaching. The conclusions regarding optimum conditions for extraction of pectin would apply to

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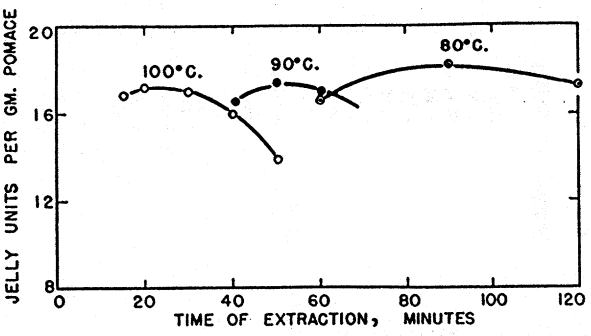

Figure 1. Effect of Time and Temperature of Extraction

both leached and nonleached pomaces). Sodium tetraphosphate was used as an extractant, although it appears that sodium hexametaphosphate (*5*) is equally effective. The following example illustrates the procedure used in these experiments.

Two hundred grams of commercial grade apple pomace (ground to 4 mesh) were leached with 3 liters of distilled water at 50° C. for 15 minutes. The leach water was removed by draining through cheesecloth, and the leached pomace was extracted with 8 grams of sodium tetraphosphate, plus sufficient dilute (1:10) hydrochloric acid to give a pH of 3.4, and sufficient distilled water to give a final weight of 2400 grams. The mixture was heated to 90° C. for 50 minutes, then cooled to 60° C., and the extract was drained through cheesecloth. The residue was pressed on a laboratory hydraulic press at 80 pounds per square inch on the cloth. The extracts were combined and filtered by means of a medium porosity filter aid. The total volume of extract was measured, and its pectin grade was determined (*14*) by preparing 65% sugar jellies. The yield of pectin, expressed as jelly units per gram of pomace, was calculated by the relation

$$\text{Jelly units/gram pomace} = \frac{\text{vol. extract} \times \text{grade of extract}}{\text{weight of pomace, grams}}$$

Figure 1 indicates the optimum periods of extraction at three temperatures. The total yield, expressed as jelly units, represents a balance between the amount of pectin extracted and the degradation of the pectin in solution. For that reason the maxima decrease with increasing temperatures. Although 90° C. and 50 minutes were chosen for laboratory scale extractions, a lower temperature and longer time are recommended for commercial operations when it is not possible to cool the extraction mixture quickly and there is considerable delay in pressing out the extracted pectin from the pomace residue. This delay lengthens the effective extraction, and for that reason it is well to start these operations several minutes before the optimum time has elapsed.

Figure 2 shows that 4 to 6% was the optimum quantity of sodium tetraphosphate at pH 3.4. Data were recently obtained which indicate that nearly maximum yields may be realized by using 3% sodium tetraphosphate at pH 3.0, or 2% sodium hexametaphosphate at pH 3.0 to 3.5. The polyphosphates gave 10 to 20% higher yields of pectin than did dilute mineral acids.

DE-ESTERIFICATION. The fact that the pH activity regions for pectase and diastase showed considerable overlapping suggested the possible advantage of performing the starch hydrolysis simultaneously with the de-esterification reaction. The properties of three enzymes must be considered—pectase, diastase, and pectinase. [The nomenclature used for the pectic enzymes is that recommended by the AMERICAN CHEMICAL SOCIETY Committee on Nomenclature of Pectin (*1*).] Pectinase is a pectin-degrading enzyme present in small amounts in commercial diastase preparations and in tomato pectase extracts. The present study was concerned primarily with the selection of conditions of pH and temperature which give efficient pectase and diastase action yet minimize pectin degradation by pectinase and by heat.

Tomato pectase was prepared from ground ripe tomatoes as previously described (*8*, *19*), and the extract was stored at 0° C. until used. The same extract was used for studies on tomato pectinase. The diastase (chiefly α-amylase) was a dried extract of mold mycelium sold under the trade name Clarase (supplied by Takamine Laboratory, Inc.).

Pectase activity was determined by continuous electrometric titration at constant pH in the presence of 0.05 *M* sodium chloride and 0.002 *M* sodium oxalate (*7*). Diastase activity was determined by the Wohlgemuth method (*21*). Pectinase activity was determined by the rate of decrease in viscosity of a 1% solution of 180-grade apple pectin, the readings being limited to the initial 15% change in relative viscosity. Under these conditions the per cent decrease in viscosity was equal to the per cent decrease in jelly grade. This enables interpretation of pectinase action on the basis of per cent change in that property of pectin (grade) commonly used for determining its commercial value. The determination of pectinase in the presence of pectase required two different substrates in order to cover the entire pH range of activity: a pectic acid substrate for pH 4.0 and above, where pectase action would cause a change in substrate (pectin to pectic acid) and a drift in pH; and a pectin substrate at pH 4.0 and below, to avoid abnormal viscosities caused by thixotrophy or by precipitation of pectic acid.

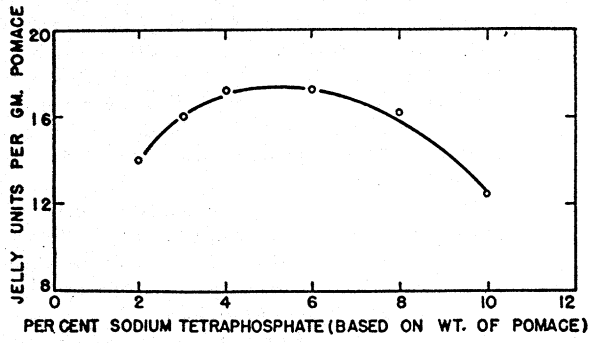

Figure 2. Effect of Concentration of Sodium Tetraphosphate

Tomato pectinase (Figure 3) was active over the range pH 3.0 to 6.5, with a narrow optimum at pH 4.8. The pectic acid degradation curve was extended to pH values below 4.0 (dotted line) by multiplying the corresponding values for pectin by the ratio between the two values at pH 4.0. The rate of degradation of pectic acid at pH 4.0 was about three times the rate for pectin at the same pH. This observation does not necessarily differ from that of Jansen and MacDonnell (*10*) regarding the relative rates of glycosidic hydrolysis of pectin and pectic acid, since our enzyme extract was a mixture of pectase and pectinase.

Although it is apparent that degradation by pectinase may be eliminated by conducting the de-esterification reaction at pH 6.5 or above and by subsequently inactivating the enzyme by heating at pH 3.0, it should be noted that the actual amount of pectinase present in tomato pectase extracts and in diastase preparations is relatively small. The amount of pectinase in the various tomato extracts studied was sufficient to cause a decrease in viscosity of 0.9 to 1.2% per hour when 1 ml. was added to 200

TABLE I. PECTIN REMOVED BY LEACHING AND SUCCESSIVE EXTRACTIONS

Fraction	Jelly Units per Gram Pomace	% of Total Jelly Units	Calculated Grade of Dry Pectin
Leach water	3.06	14	260
1st extract	17.25	79	240
2nd extract	1.20	6	90
3rd extract	0.25	1	90 (?)

ml. of 1% pectin solution at pH 4.0 and 30° C. This ratio of enzyme to substrate is the same as that used in the de-esterification of pomace extracts in 1 hour at pH 6.5 and 30° C. The amount of pectinase contributed by the diastase (Clarase) under these conditions would cause a loss of about 0.001% per hour, calculated on the basis of the ratio of enzyme to substrate used in starch removal.

The diastase preparation has a much broader pH range than pectinase and may be used at a pH at which the latter enzyme is inactive (6.5 to 7.5). Although the relative activity of the diastase in this pH range is only 57 to 28% of the maximum, the amount of diastase preparation required is small—about 3 to 10 grams per 100 gallons of apple pomace extract. In the pH range 3.2 to 3.5, commonly used for starch hydrolysis of pomace extracts, diastase reacts at only 4 to 12% of its maximum velocity.

Tomato pectase is only slightly less active at pH 6.5 than at the optimum pH of 7.5, but, because of the greater heat degradation of pectin at the latter pH at temperatures no higher than 40° C., it appears desirable to conduct the de-esterification at pH 6.5. Tomato pectase activity is also affected by salt concentration (7). Concentrated pomace extracts prepared by polyphosphate extraction contain sufficient cations in solution to give full pectase activity, but extracts prepared by heating with dilute mineral acids may require additional salt to provide efficient pectase action.

The increase in pectase activity with increase in substrate concentration (7) would provide a 5% saving in quantity of enzyme in de-esterifying a concentrated (2%) instead of a dilute (0.8%) pomace extract.

The temperature at which the de-esterification is conducted is extremely important. The principal considerations are the increase in pectase activity and the extent of heat degradation of the pectin at higher temperatures. The Q_{10} value for tomato pectase is approximately 1.4 within the range studied (7). At temperatures above 40° C. the inactivation of the enzyme becomes a factor in the over-all rate for a given time interval. For that reason it is better to compare the extent of reaction per unit volume of enzyme for a given time. On the basis of a 1-hour reaction the observed amounts of de-esterification at temperatures of 30°, 40°, 50°, and 60° C. were in the ratio of 1.0, 1.42, 1.68, and 1.36, respectively.

Table II. Effect of pH on Yields by Polyphosphate Extraction of Apple Pomace

(90° C., 50 min., 4% sodium tetraphosphate)

pH	Jelly Units per Gram Pomace
2.5	17.20
3.0	17.30
3.4	17.45
4.0	16.90

Table III. Heat Degradation of Pomace Extract at Various Temperatures and pH Values

Condition of Heating		Time, Hr.	Grade of Extract	Decrease in Grade, %/Hour
° C.	pH			
Control, not heated		...	1.82	...
30	6.5	4	1.75	0.95
40	6.5	4	1.55	3.7
50	6.5	2	1.44	10.4
60	6.5	1	1.30	28.6
40	6.5	4	1.55	3.7
40	7.0	4	1.39	5.9
40	7.5	4	1.26	7.7

Table IV. Inactivation of Tomato Pectinase in Presence of Pectin

Condition of Heating		Time, Min.	% Activity Retained
° C.	pH		
Control—not heated		...	100.0
50	4.0	30	21.0
	4.0	30	6.1
	4.0	30	2.5
65	3.5	10	1.3
65	3.5	30	0.5

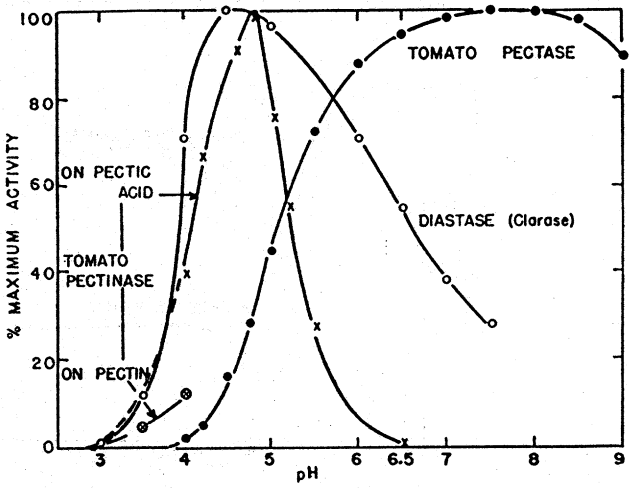

Figure 3. pH-Activity Relations of Pectase, Pectinase, and Diastase

The rate of heat degradation of a 1.82-grade pomace extract is recorded in Table III in terms of per cent decrease in grade per hour. Two facts are apparent: The rate of degradation was increased by an increase in pH from 6.5 to 7.5, and the amount of degradation at pH 6.5 increased about 3.5-fold for each 10° C. rise in temperature. In view of these results it does not appear economical to conduct the enzyme de-esterification at a temperature above 40° C. or at a pH above 6.5.

In general, it is advisable to heat-inactivate the pectinase in the de-esterification mixture, because the enzyme is precipitated with the low ester pectin and may be carried through to the final dried product. The extent of pectinase inactivation in the presence of pectin was determined by heating mixtures of two parts of tomato pectase extract and three parts of pomace extract. Aliquots of the heated mixtures were tested for pectinase activity at pH 4.0 on pectin substrate by the method previously described. The data (Table IV) indicate that temperatures of 65° C. or higher are required to destroy pectinase. An examination of the data for pH 4.0 and 3.5 indicates that lower pH values would be even more effective. Purification of the low ester pectin by washing with acidified alcohol eliminated about 95% of the pectinase present. Thus either procedure, or a combination of both, may be used to eliminate pectinase from the final dried product. Pectase and diastase are effectively eliminated by conditions used for pectinase inactivation. In fact, tomato pectase is readily destroyed by alcohol at room temperature.

SUGGESTED PROCEDURE FOR PREPARATION

A satisfactory procedure for the preparation of low ester pectin may be illustrated by the following example:

Eighty pounds of dried apple pomace were placed in a 200-gallon stainless steel tank provided with steam coil and a propeller-type agitator. Hot tap water was added to give a total volume of 150 gallons, and the mixture was heated to 80° C. Three pounds of sodium tetraphosphate and 830 ml. of dilute (1:5) sulfuric acid were added (final pH was 3.1).

The mixture was allowed to stand for 60 minutes at 80° C. and then cooled to 60° C. by circulating cold water through the steam coil in the extraction tank. The pectin extract was pressed from the pomace by means of a hydraulic press and then filtered through a plate-and-frame filter press using a medium grade of diatomaceous filter aid. The filtered extract (110 gallons) was concentrated to 39 gallons in a forced-circulation vacuum evaporator.

The pectin concentrate was placed in a 50-gallon tank equipped with an air-driven stirrer. An aliquot of the pectin concentrate

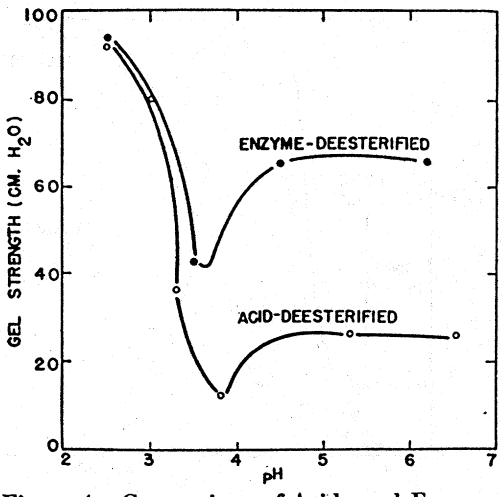

Figure 4. Comparison of Acid- and Enzyme-De-esterified Pectins

was assayed for pectin methyl ester by the following modification of the previously described method (*8*):

One hundred ml. (103 grams) of concentrate were weighed into a 600-ml. beaker and diluted to 500 ml. The solution was neutralized to pH 7.50, and 50 ml. of tomato pectase extract (pH 7.5) were added. After 30 minutes the mixture was again titrated to pH 7.5; titration required 7.7 cc. of 0.5 *N* NaOH. In the present experiment it was desired to hydrolyze 50% of the enzyme labile methyl ester groups. This would require

$$\frac{7.70 \times 37.85 \times 39 \times 0.50}{4} = 1420 \text{ ml. of } 2.0\ N \text{ NaOH}$$

The pectin concentrate was adjusted to pH 6.50 and 40° C., and 2 liters of tomato pectase extract (pH 6.50) and 10 grams of diastase (Clarase) were added. The mixture was stirred and the pH maintained at 6.50 by the continuous addition of 2.0 *N* NaOH from a large buret. The diastase reaction reached completion (negative starch-iodine test) in 30 minutes. The de-esterification reaction was allowed to proceed until the required amount of 2.0 *N* NaOH (1420 ml.) had been consumed (38 minutes). The mixture was immediately acidified to pH 4.0 by adding 800 ml. of 1:5 sulfuric acid and then heating to 65° C. for 20 minutes. The low ester pectin was precipitated with 1.25 volumes of 80% alcohol and pressed on a hydraulic press. The pressed precipitate was disintegrated and covered with 10 gallons of 95% alcohol and allowed to stand for 2 hours. The alcohol was again removed by pressing. The low ester pectin was dried 16 hours in a forced draft air oven at 70° C. and then ground to 60 mesh in a hammer mill. The yield was 5.0 pounds, or 6.25% of the weight of the apple pomace used. The product analyzed 4.2% methoxyl and was calculated to be 33% esterified.

DISCUSSION

The proposed process for low ester pectin by enzyme de-esterification requires only minor changes in the method of manufacture commonly used for apple pectin. The use of polyphosphates facilitates the extraction of pectin from apple pomace and also provides a calcium-sequestering agent for subsequent de-esterification. By combining starch hydrolysis and enzyme–de-esterification procedures, it is possible to reduce the steps in the process, and the more favorable pH effects a considerable saving in the amount of diastase required. Degradation is reduced to a minimum by the proper selection of pH and temperature for de-esterification and for subsequent heat-inactivation of enzymes.

The previous publication (*9*) on the preparation of enzyme-de-esterified pectin stressed the necessity for removing calcium and other polyvalent cations from the final product. More recently it has been observed that precipitation at pH 4.0, as in the example cited, improved its dispersibility and minimized the need for extensive purification. Low ester pectins which have been precipitated at pH 4.0 or above are usually soluble and readily dispersible in water. If the source of water used for pectin extraction contains large amounts of calcium or magnesium salts, it may be necessary to deionize by ion-exchange resins.

The product prepared by the present process was evaluated for the purpose of preparing calcium pectinate gels. Gels were prepared by the previously described method (*9*) except that sugar was omitted from the formula and the pH was varied by the addition of dilute hydrochloric acid or dilute sodium hydroxide. Gels were prepared in which the amount of added calcium salt was varied to determine the optimum calcium concentration and maximum gel strength at each selected pH. In all cases the concentration of low ester pectin in the final gel was 1%.

In Figure 4 gel strength is plotted against pH. The curve for an acid–de-esterified pectin prepared from a source material of comparable quality (200-grade apple pectin) is presented for comparison. The methoxyl value (5.2%) and degree of esterification (38%) of the acid-prepared product are close to the values for the enzyme–de-esterified product. The enzyme–de-esterified low ester pectin is equal in gel strength to the acid-deesterified product at low pH values and is definitely superior at pH values above 3.5. The data cover the pH range commonly encountered in food products.

The difference in properties of acid- and enzyme-de-esterified pectins is due in part to the difference in distribution of carboxyl groups along the polygalacturonide chain, as indicated by electrophoretic patterns (*18*) and by qualitative differences in viscosity-pH relations (*17*).

Low ester pectins may be utilized for a wide variety of food and nonfood uses. Various known and suggested uses have already been described in recent publications (*2, 3, 6, 9, 11, 12, 15, 16*).

Part of the wartime increase in pectin production facilities may be utilized in the postwar period for the production of low ester pectins. The enzyme process provides an economical method for preparing low ester pectin.

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